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Herpes Simplex Virus Type 1 Suppresses the Interferon Signaling Pathway by Inhibiting Phosphorylation of STATs and Janus Kinases during an Early Infection Stage

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We examined the influence on the interferon (IFN) signaling pathway of infection with herpes simplex virus type 1 (HSV-1) strain VR3. Data from reporter gene assays showed that expression of both type I and type II IFN-inducible genes was dramatically suppressed during the early stage of HSV-1 infection (2 to 3 h postinfection). During these periods, phosphorylation levels of janus kinases (JAKs) and STATs did not increase after treatment of HSV-1-infected FL cells with IFN-α or IFN-γ, although cellular protein levels of the JAKs and the STATs were not significantly changed. In contrast, the inhibitory effect of HSV-1 on phosphorylation of STAT1 was not observed in U937 cells, which show resistance to steady-state accumulation of RNA for HSV-1 immediate-early genes. The phosphorylation of STAT1 in FL cells was not inhibited by infection with a UV-inactivated virus. These results indicate that viral gene expression or viral protein production is necessary for the inhibition of phosphorylation by HSV-1.

Key Words: herpes simplex virus type 1; interferon; JAK/STAT pathway; STAT1; STAT2; Jak1; Jak2; Tyk2; tyrosine phosphorylation.

INTRODUCTION

Interferon (IFN) plays an important role in the defense of a cell against viral infection. It works through the induction or activation of antiviral enzymes such as the 2′,5′-oligoadenyl synthetase/RNase L system, the double-stranded RNA-activated protein kinase (PKR), and the MxA protein (Fujii, 1994; Kerr and Stark, 1992; Samuel, 1991; Sen and Ransohoff, 1993). The intracellular signal transduction pathway of IFN is known to be the JAK/STAT pathway (Darnell *et al.,* 1994; Goodbourn *et al.,* 2000; Stark *et al.,* 1998). The binding of IFNs to their specific receptors stimulates the phosphorylation, and therefore the activation, of janus kinases (JAKs), which in turn activate STAT1 and/or STAT2 through phosphorylation. IFN-α induces the transcription factor ISGF3, which consists of three components: phosphorylated STAT1, phosphorylated STAT2, and p48 (also called IRF-9 or ISGF3γ). Similarly, IFN-γ induces the transcription factor GAF, a homodimer of phosphorylated STAT1α. The induced ISGF3 complex specifically binds to the ISRE (interferon-stimulated response element), and the GAF complex specifically binds to the GAS (γ activation sequence). Both ISRE and GAS are located in the promoter region of the IFN-inducible genes and promote the genes' expression.

Viruses, including both DNA and RNA viruses, can inhibit such IFN-induced host defense mechanisms by various strategies. Many viruses are known to suppress IFN-induced antiviral activity by inhibiting the JAK/STAT signaling pathway (Alcami and Koszinowski, 2000; Goodbourn *et al.,* 2000), which is considered to be an important strategy for escaping the host defense system. A review of recent reports indicated that the mechanisms of inhibition are varied (Alcami and Koszinowski, 2000; Goodbourn *et al.,* 2000). Some viruses reduce the constitutive or basal levels of particular molecules that contribute to the JAK/STAT pathway; for example, human cytomegalovirus (HCMV) reduces the basal levels of Jak1 and p48, although the mechanism by which it does this is unknown (Miller *et al.,* 1998, 1999), and simian virus 5 and mumps virus degrade STAT1 (Didcock *et al.,* 1999; Fujii *et al.,* 1999; Yokosawa *et al.,* 1998). Inactivation of components of the JAK/STAT pathway by particular viral products has also been demonstrated following infection by several types of virus. For example, T antigen of murine polyoma virus and E7 protein of human papillomavirus 16 bind to Jak1 and p48, respectively (Barnard and McMillan, 1999; Weihua *et al.,* 1998). Recent studies indicate that the human herpes simplex virus type 1 (HSV-1) activates IFN-inducible genes in the absence of *de novo* cellular protein synthesis (Mossman *et al.,* 2001; Nicholl *et al.,* 2000). This response is indicated to be suppressed by HSV-1 infection when viral genes are expressed (Mossman *et al.,* 2001). The evidence sug-

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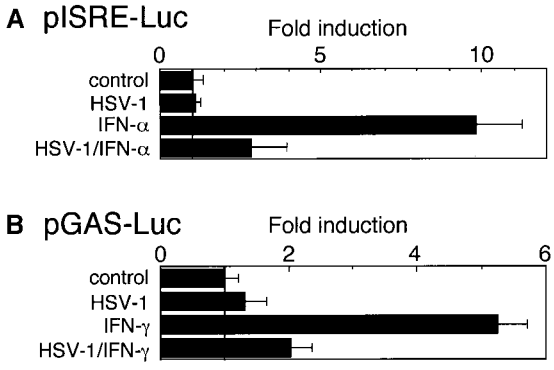

FIG. 1. Effect of HSV-1 infection on induction of IFN-inducible genes determined by a reporter gene assay. FL cells were transfected with reporter plasmids containing luciferase reporter genes linked to IFN-responsive enhancers, pIRSE-Luc for IFN-α stimulation (A) or pGAS-Luc for IFN-γ stimulation (B), plus pRL-TK as an internal reference of transfection efficacy. The transiently expressed transfectants were infected with HSV-1 at m.o.i. 5 or left uninfected as control. After 2 h incubation, 1000 IU/ml IFN-α or IFN-γ was added, and then the cells were incubated for another 4 h. The cells were lysed, and the luciferase activities in the resulting cell lysates were measured. All experiments were carried out in triplicate. The results are expressed as fold induction, which is relative to the values obtained from control experiments with neither HSV-1 infection nor IFN treatment.

gested that HSV-1 has a mechanism for suppression of the IFN signaling pathway. Here we report a novel mechanism for the suppression of the IFN signaling pathway during an early stage of infection by HSV-1.

RESULTS

Suppression of IFN-inducible gene expression during an early stage of HSV-1 infection

We examined the effect of HSV-1 infection on IFN-inducible gene expression by a reporter gene assay using luciferase as the reporter. To examine the response to IFN-α, five tandem repeats of ISRE were used as an enhancer element of the firefly luciferase gene. We used a dual luciferase assay system using the *Renilla* luciferase gene controlled under the HSV thymidine kinase promoter as a control, in order to determine the specific changes of the reporter activity by IFN and HSV-1 treatment and to correct the transfection efficacy of the plasmid DNA. Treatment with 1000 IU/ml IFN-α for 4 h increased firefly luciferase activity about 10-fold, compared with the untreated control. Although HSV-1 infection itself did not alter firefly luciferase activity at 6 h postinfection (p.i.), the induction of the firefly luciferase activity by IFN-α treatment was dramatically suppressed by infection with HSV-1 at a multiplicity of infection of 5 (m.o.i. 5) 2 h prior to the IFN-α treatment (Fig. 1A). To examine IFN-γ-induced gene expression, four tandem repeats of the GAS element were used as an enhancer element for the firefly luciferase gene. Similar to what was seen for IFN-α, IFN-γ treatment enhanced the firefly luciferase activity about 5-fold compared to the untreated control, and the enhancement was strongly suppressed by HSV-1 infection 2 h prior to the IFN-γ treatment (Fig. 1B). The results indicate that HSV-1 suppresses the signal transduction of both type I IFN (IFN-α/β) and type II IFN (IFN-γ), and therefore the subsequent IFN-induced gene expression, during an early stage of infection.

Protein levels of STATs, p48, and janus kinases during HSV-1 infection

To determine the suppression mechanism of IFN-inducible gene expression by HSV-1 infection, first, we investigated changes of protein levels of STAT1, STAT2, p48, Jak1, Jak2, and Tyk2 in FL cells during HSV-1 VR3 infection by Western blotting (Fig. 2). At late stage of infection (16 h p.i.), protein levels of STAT2 and Jak1 were dramatically decreased. On the other hand, STAT1α and β, p48, Jak2, and Tyk2 did not show significant changes even at 16 h p.i. The results indicate that the protein levels of all molecules tested did not show any significant changes during early stage of infection when the suppression of IFN-inducible gene expression was seen as shown in Fig. 1.

Inhibition of IFN-induced phosphorylation of STAT1α and STAT2 by HSV-1 infection

Protein levels of molecules contributing IFN signaling pathway did not change during the early infection stage as shown above (Fig. 2), so we analyzed the activation step, namely phosphorylation, of the signal transduction molecules. First IFN-induced tyrosine (Tyr) 701 phosphorylation of STAT1α in HSV-1-infected cells was ana-

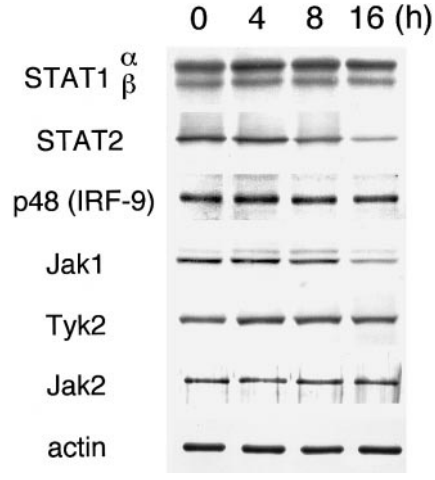

FIG. 2. Changes of protein levels of STAT1, STAT2, p48, Jak1, Tyk2, and Jak2 in FL cells by HSV-1 infection. FL cells were infected with the HSV-1 strain VR3 at m.o.i. 5. At various periods, the cells were lysed, and the lysates (30 μg per lane) were applied to SDS–PAGE [on a 6% (w/v) acrylamide gel, except for p48 and actin on a 12.5% (w/v) gel] and analyzed by Western blotting. Actin was determined as a control for protein loading.

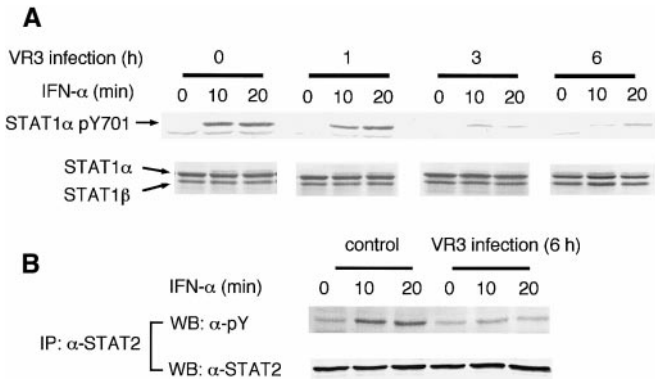

FIG. 3. Inhibition of IFN-α-induced tyrosine phosphorylation of STAT1α and STAT2 by HSV-1 infection in FL cells. (A) FL cells were infected with the HSV-1 strain VR3 for various times at m.o.i. 5. At each period, the cells were treated with IFN-α (1000 IU/ml) for 10 or 20 min and then lysed. The lysate (30 μg per lane) was applied to SDS–PAGE on a 6% (w/v) polyacrylamide gel and analyzed by Western blotting using anti-Tyr-phosphorylated STAT1 and anti-STAT1 antibodies. (B) FL cells were infected with HSV-1 VR3 for 6 h at m.o.i. 5. The infected cells and uninfected control cells were treated with IFN-α (1000 IU/ml) for 10 or 20 min and then lysed. STAT2 proteins were precipitated from the lysate with anti-STAT2 antibody and then analyzed by Western blotting using anti-phosphotyrosine (pY) antibody and anti-STAT2 antibody.

lyzed by Western blotting. Both IFN-α- and IFN-γ-induced phosphorylation of STAT1α was significantly inhibited 3 h p.i. of FL cells with HSV-1 (Figs. 3A and 4B). However, protein levels of STAT1 did not show a significant change during these periods. Similar results for the inhibition of STAT2 phosphorylation, which was determined by immunoprecipitation with anti-STAT2 antibody followed by Western blotting using anti-phosphotyrosine antibody, were observed in HSV-1-infected FL cells after treatment with IFN-α (Fig. 3B).

To investigate whether the suppression of STAT phos-

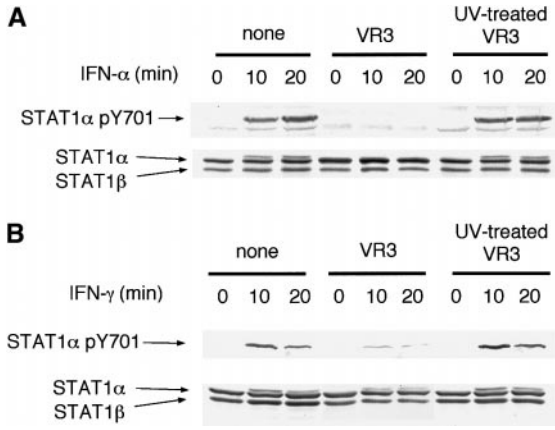

FIG. 4. Effect on IFN-induced STAT1 phosphorylation by UV-inactivated HSV-1 infection in FL cells. FL cells were infected with native or UV-inactivated HSV-1 VR3 for 6 h at m.o.i. 5 and then treated with 1000 IU/ml IFN-α (A) or IFN-γ (B) for 10 or 20 min. The resulting cell lysate was analyzed by Western blotting using anti-Tyr-phosphorylated STAT1 and anti-STAT1 antibodies as shown in Fig. 3.

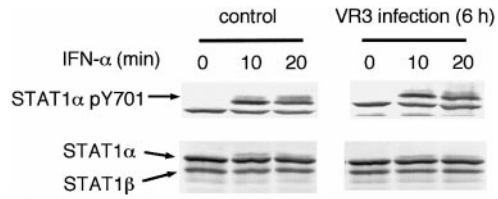

FIG. 5. Effect on IFN-induced STAT1 phosphorylation by HSV-1 infection in U937 cells. U937 cells were infected with HSV-1 for 6 h at m.o.i. 5 and then treated with IFN-α (1000 IU/ml) for 10 or 20 min. The resulting cell lysate was analyzed by Western blotting using anti-Tyr-phosphorylated STAT1 and anti-STAT1 antibodies as shown in Fig. 3.

phorylation is dependent on the expression of HSV-1 genes, FL cells were infected with UV-inactivated HSV-1. No production of virus particles and no cytopathic effects of the UV-inactivated virus were found in FL cells or in Vero cells as an indicator (data not shown). No inhibition of STAT1α phosphorylation was found in FL cells infected with the UV-inactivated HSV-1 after treatment with either IFN-α or IFN-γ (Figs. 4A and 4B). Furthermore, the inhibition of IFN-α-induced STAT1α phosphorylation was not observed in the human monocytic cell U937 after infection of HSV-1 for 6 h at m.o.i. 5 (Fig. 5). It has been reported that U937 cells have a mechanism that protects them against HSV-1 at the step of viral replication, but not at the infection or penetration steps. In U937 cells, viral DNA is transported to the cell nucleus, but steady-state accumulation of viral RNA for immediate-early (IE) genes does not occur (Stewart *et al.,* 1992; Tenney and Morahan, 1987, 1991). These results indicate that HSV-1 inhibits the phosphorylation, and therefore the activation, of STAT1α and STAT2 in a manner that is dependent on viral gene expression or viral protein production.

To confirm the specificity of the inhibitory effect on STAT phosphorylation, other protein phosphorylation was examined. Heat-shock-induced phosphorylation of heat shock factor 1, which is a transcription factor for stress-responsible genes such as heat shock protein genes (Wu, 1995), was not altered by HSV-1 infection until at least 6 h p.i. (data not shown). Double-stranded RNA [poly(rI) · poly(rC)]-induced hyperphosphorylation on several C-terminal serine and threonine residues of interferon-regulatory factor 3, which is a component of the transcriptional activating complex for IFN-β production (Lin *et al.,* 1998; Weaver *et al.,* 1998), was not suppressed by HSV-1 infection until at least 6 h p.i. (data not shown).

Inhibition of IFN-induced phosphorylation of JAKs by HSV-1 infection

Since STAT1α and STAT2 are directly phosphorylated by IFN-activated JAKs, we suspected that the suppression of STAT phosphorylation might be caused by the downregulation of JAK activity. We therefore examined the effect of HSV-1 infection on the phosphorylation, and

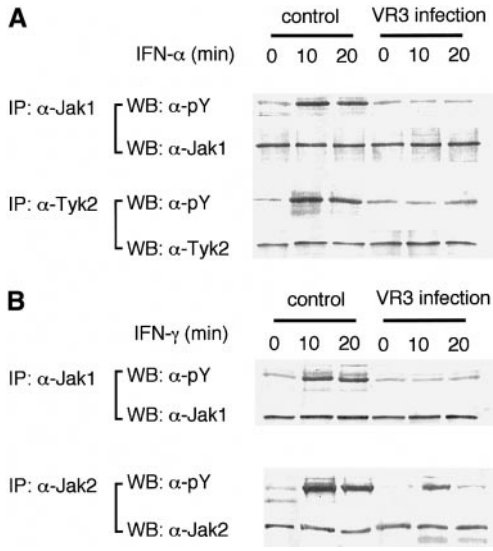

FIG. 6. Downregulation of IFN-α-induced Jak1 and Tyk2 phosphorylation (A) and IFN-γ-induced Jak1 and Jak2 phosphorylation (B) by HSV-1 infection. FL cells were infected with HSV-1 VR3 for 6 h at m.o.i. 5, and then treated with IFN-α (1000 IU/ml) or IFN-γ (1000 IU/ml) for 10 or 20 min. The kinase proteins were immunoprecipitated from the cell lysate with specific antibodies and analyzed by SDS–PAGE on a 6% polyacrylamide gel and followed by Western blotting using anti-phosphotyrosine (pY) antibody and anti-Jak1, anti-Tyk2, or anti-Jak2 antibody.

therefore the activation, of JAKs by IFN estimated by immunoprecipitation with respective JAK-specific antibody followed by Western blotting with anti-phosphotyrosine antibody. Jak1 and Tyk2 phosphorylation induced by IFN-α was downregulated 3 h after HSV-1 infection (Fig. 6A). Similarly, IFN-γ-induced phosphorylation of Jak1 and Jak2 was also downregulated after HSV-1 infection for 3 h (Fig. 6B). However, cellular protein levels of these JAKs, which were estimated by Western blotting of the whole-cell extracts (Fig. 2) and the immunoprecipitates by using the respective JAK-specific antibody (Fig. 6), were not altered under these conditions of HSV-1 infection and IFN treatment. We conclude that impaired phosphorylation of JAKs results in the suppression of STAT phosphorylation.

DISCUSSION

In this study, we show that HSV-1 inhibits the IFN signaling pathway by the suppression of IFN-induced phosphorylation of STATs and JAKs early in the process of infection (2 to 3 h p.i.). Since STATs are directly phosphorylated by JAKs (Darnell *et al.,* 1994; Stark *et al.,* 1998), the inhibition of STAT phosphorylation is thought to be due to a failure of JAK activation. Indeed, the phosphorylation of JAKs was poor in FL cells infected with HSV-1. The inhibition of phosphorylation seems to require the expression of HSV-1 genes and the subsequent production of viral proteins, although the expression of the HSV-1 genes did not affect the protein levels of the JAK and STAT molecules in the periods of HSV-1 infection used during this experiment. This evidence is the first report of suppression of JAK phosphorylation by a DNA virus. HSV-1 seemed to have a broad inhibitory function, in that it affected all types of JAKs investigated here.

The JAK/STAT signaling pathway in response to IFN stimulates transcription of IFN-inducible genes and leads to the induction of antiviral actions (Darnell *et al.,* 1994; Goodbourn *et al.,* 2000; Stark *et al.,* 1998). Many viruses have evolved several different anti-IFN mechanisms. These mechanisms are divided into two categories: first is the production of viral proteins that counteract the antiviral proteins induced by IFNs, and second is through the degradation of particular protein components that contribute to IFN signaling. In *Herpesviridae,* the ICP 34.5 protein of HSV-1 is an example of the first category. It counteracts PKR-mediated phosphorylation to prevent the establishment of an antiviral state (He *et al.,* 1997). For an example of the second category in *Herpesviridae,* HCMV reduces basal cellular levels of Jak1 and p48, thereby preventing the transduction of JAK/STAT signals (Miller *et al.,* 1998, 1999). Here, we propose a new third category of anti-IFN mechanisms that cause dysfunction in the IFN signaling pathway by the suppression of JAK phosphorylation. Infection with Sendai virus (SeV), and RNA virus, also results in a slight reduction of JAK phosphorylation (Komatsu *et al.,* 2000). However, it is still unknown whether the inhibitory mechanism of SeV is similar to that of HSV-1 as presented in this study. At a late stage of HSV-1 infection, we have observed that basal levels of Jak1 and STAT2 proteins decrease in FL cells (Fig. 2). This observation at a late infection stage may be a phenomenon similar to that reported for HCMV (Miller *et al.,* 1998, 1999), falling into the second category described above. Therefore, we propose that the JAK/STAT signal transduction pathway is strongly impaired by multiple mechanisms during HSV-1 infection, with downregulation of JAK phosphorylation during the early infection stage and degradation of particular components of the signaling pathway late in the infection process.

Based on our observations that UV-inactivated virus cannot suppress the phosphorylation of STATs in FL cells (Fig. 4), and that the suppression of the JAK/STAT pathway does not occur in U937 cells (Fig. 5), which show resistance to HSV-1 replication, we have concluded that impaired or reduced phosphorylation of JAKs seemed to require HSV-1 gene expression or production of an HSV-1 protein(s). A newly synthesized viral protein(s) should therefore contribute to this event. Recent reports showed that HSV-1 activates IFN-inducible genes in the absence of *de novo* cellular protein synthesis (Mossman *et al.,* 2001; Nicholl *et al.,* 2000). This response is seen only when viral gene expression is inhibited. So a newly synthesized viral protein(s) may function as an inhibitor of the IFN-inducible gene expression. The observation

agreed well with our results presented here. Furthermore, Mossman *et al.* (2000) also showed that an IE gene product, ICP0 protein, should contribute to the relative resistance of HSV-1 to IFN, because HSV-1 mutants lacking the ICP0 gene are hypersensitive to IFN. The human monocytic cell line U937 is an interesting system, because the primary block in HSV-1 replication in U937 cells occurs after transport of the viral DNA to the cell nucleus but prior to steady-state accumulation of viral RNA for IE genes (Stewart *et al.,* 1992; Tenney and Morahan, 1987, 1991). Stewart *et al.* (1992) also indicated that the resistance to HSV-1 replication in U937 cells is related to transcriptional events with regard to the ICP0. The dysfunction in ICP0 activity in U937 cells may be involved in the lack of IFN-induced Tyr phosphorylation of STAT1α. The inhibition should result in the suppression of the initiation step of the HSV-1 replication process in early stages of infection. We believe that the inhibitory mechanism for decreasing IFN signaling that we present in this study contributes to allowing HSV-1 to elude this first barrier of the host defense and therefore to be able to proceed with its replication even in the presence of IFN. We are currently examining what viral molecules might participate in the inhibition of JAK and STAT phosphorylation and how they work.

MATERIALS AND METHODS

Cell culture, virus infections, and IFN treatment

Human amnion cell FL, human monocytic cell U937, and Vero cells were routinely cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum. HSV-1 strain VR3 was obtained from the American Type Culture Collection (Rockville, MD). UV-inactivated virus was prepared by irradiation with a 15-W UV lamp (GL-15; Toshiba, Tokyo, Japan) at a distance of 10 cm for 5 min. The inactivation was confirmed by a plaque formation assay using Vero cells and FL cells as indicator cells.

All HSV-1 infections were done at m.o.i. 5. IFN-α and IFN-γ were purchased from Serotec (Oxford, UK) and Genzyme (Cambridge, MA), respectively. Both were used at a final concentration of 1000 IU/ml.

Antibodies

Rabbit polyclonal antibodies against STAT1 p84/p91 (E-23), STAT2 (C-20), and Jak2 (M-126) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit antibodies against Jak1 and Tyk2 were from Upstate Biotechnology (Lake Placid, NY). Anti-Tyr^{701}-phosphorylated STAT1 antibody was from BioSource International (Camarillo, CA). Mouse anti-p48 monoclonal antibody (clone 6) and alkaline phosphatase-conjugated recombinant anti-phosphotyrosine antibody (RC20) were from Transduction Laboratories (San Diego, CA). Mouse monoclonal antibody to actin (C4) was from Chemicon (Temecula, CA).

Western blotting

Cells were lysed with 1% Nonidet P-40, 120 mM NaCl, 1 mM dithiothreitol, 10% glycerol, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 1 mM NaF, and 20 mM HEPES–NaOH, pH 7.5, and the lysates were centrifuged at 10,000 *g* for 10 min at 4°C. The supernatants were recovered, and the protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were carried out as described previously (Yokosawa *et al.,* 1998; Yokota *et al.,* 1999).

Immunoprecipitation analysis

Cellular proteins were solubilized by RIPA buffer (1% Nonidet P-40, 0.1% sodium deoxycholate, 100 mM NaCl, 4 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.2 mTIU/ml aprotinin, 1 mM Na_3VO_4, 1 mM NaF, and 50 mM HEPES–NaOH, pH 7.5). The cell lysate and 5 μg antibody were mixed and incubated at 4°C for 1 h. Protein G–Sepharose 4B (Amersham Pharmacia, Uppsala, Sweden) was added to the mixture and incubated at 4°C overnight on a rotator. The resin was washed with the RIPA buffer four times and then the bound materials were solubilized by boiling with SDS–PAGE sample buffer and applied to SDS–PAGE on a 6% (w/v) polyacrylamide gel, followed by Western blotting analysis as above.

Reporter gene assay using luciferases

Expression of IFN-inducible genes was determined by a dual luciferase reporter assay by transiently transfecting reporter plasmids containing either the ISRE or the GAS enhancer element. The reporter plasmids, pISRE-Luc and pGAS-Luc (PathDetect in Vivo Signal Transduction Pathway *cis*-Reporting Systems), were purchased from Stratagene (La Jolla, CA). pISRE-Luc and pGAS-Luc harbor the enhancer elements $(TAGTTTCACTTTCCC)_5$ and $(AGTTTCATATTACTCTAAATC)_4$, respectively, upstream of the firefly luciferase gene. FL cells were plated onto a 24-well plate at approximately 30% confluency the day before transfection. Reporter plasmids (1 μg) and reference plasmids (0.1 μg of pRL-TK vector, harboring the HSV thymidine kinase promoter just upstream of *Renilla* luciferase; Promega, Madison, WI) were mixed and transfected into the FL cells using Superfect transfection reagent (Qiagen, Hilden, Germany) according to the manufacturer's instruction manual. After cultivation for 36 h, the transfectants were infected with HSV-1 strain VR3 at m.o.i. 5 or not infected. After 2 h incubation, IFN-α (for pISRE-Luc transfectants) or IFN-γ (for pGAS-Luc transfectants) was added at a final concentration of 1000 IU/ml and further incubated for 4 h. The cells were

lysed, and the firefly luciferase and *Renilla* luciferase activities in the lysate were measured using the Dual-Luciferase Reporter Assay System (Promega). The experiments were performed in triplicate. Reporter activity was calculated as the ratio of firefly luciferase activity to *Renilla* luciferase activity. The results are expressed as fold induction, which is the value relative to the value of control experiments with neither HSV-1 infection nor IFN treatment.

REFERENCES

Alcami, A., and Koszinowski, U. H. (2000). Viral mechanisms of immune evasion. *Trends Microbiol.* **8,** 410–418.

Barnard, P., and McMillan, N. A. (1999). The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-α. *Virology* **259,** 305–313.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994). Jak–STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264,** 1415–1421.

Didcock, L., Young, D. F., Goodbourn, S., and Randall, R. E. (1999). The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. *J. Virol.* **73,** 9928–9933.

Fujii, N. (1994). 2-5A and virus infection. *In* "Progress in Molecular and Subcellular Biology" (W. E. G. Müller and H. C. Schröder, Eds.), pp. 151–175. Springer-Verlag, Berlin.

Fujii, N., Yokosawa, N., and Shirakawa, S. (1999). Suppression of interferon response gene expression in cells persistently infected with mumps virus, and restoration from its suppression by treatment with ribavirin. *Virus Res.* **65,** 175–185.

Goodbourn, S., Didcock, L., and Randall, R. E. (2000). Interferons: Cell signalling, immune modulation, antiviral responses and virus countermeasures. *J. Gen. Virol.* **81,** 2341–2364.

He, B., Gross, M., and Roizman, B. (1997). The $\gamma_1$34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1α to dephosphorylate the α subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc. Natl. Acad. Sci. USA* **94,** 843–848.

Kerr, I. M., and Stark, G. R. (1992). The antiviral effects of the interferons and their inhibition. *J. Interferon Res.* **12,** 237–240.

Komatsu, T., Takeuchi, K., Yokoo, J., Tanaka, Y., and Gotoh, B. (2000). Sendai virus blocks alpha interferon signaling to signal transducers and activators of transcription. *J. Virol.* **74,** 2477–2480.

Lin, R., Heylbroeck, C., Pitha, P. M., and Hiscott, J. (1998). Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *Mol. Cell. Biol.* **18,** 2986–2996.

Miller, D. M., Rahill, B. M., Boss, J. M., Lairmore, M. D., Durbin, J. E., Waldman, J. W., and Sedmak, D. D. (1998). Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway. *J. Exp. Med.* **187,** 675–683.

Miller, D. M., Zhang, Y., Rahill, B. M., Waldman, W. J., and Sedmak, D. D. (1999). Human cytomegalovirus inhibits IFN-α-stimulated antiviral and immunoregulatory responses by blocking multiple levels of IFN-α signal transduction. *J. Immunol.* **162,** 6107–6113.

Mossman, K. L., Macgregor, P. F., Rozmus, J. J., Goryachev, A. B., Edwards, A. M., and Smiley, J. R. (2001). Herpes simplex virus triggers and then disarms a host antiviral response. *J. Virol.* **75,** 750–758.

Mossman, K. L., Saffran, H. A., and Smiley, J. R. (2000). Herpes simplex virus ICP0 mutants are hypersensitive to interferon. *J. Virol.* **74,** 2052–2056.

Nicholl, M. J., Robinson, L. H., and Preston, C. M. (2000). Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. *J. Gen. Virol.* **81,** 2215–2218.

Samuel, C. E. (1991). Antiviral actions of interferon. Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. *Virology* **183,** 1–11.

Sen, G. C., and Ransohoff, R. M. (1993). Interferon-induced antiviral actions and their regulation. *Adv. Virus Res.* **42,** 57–102.

Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998). How cells respond to interferons. *Annu. Rev. Biochem.* **67,** 227–264.

Stewart, D. R., Anaraki, F., and Leary, K. (1992). Analysis of the basis for persistence of herpes simplex virus type 1 in undifferentiated U937 cells. *Viral Immunol.* **5,** 173–184.

Tenney, D. J., and Morahan, P. S. (1987). Effects of differentiation of human macrophage-like U937 cells on intrinsic resistance to herpes simplex virus type 1. *J. Immunol.* **139,** 3076–3083.

Tenney, D. J., and Morahan, P. S. (1991). Differentiation of the U937 macrophage cell line removes an early block of HSV-1 infection. *Viral Immunol.* **4,** 91–102.

Weaver, B. K., Kumar, K. P., and Reich, N. C. (1998). Interferon regulatory factor 3 and CREB-binding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. *Mol. Cell. Biol.* **18,** 1359–1368.

Weihua, X., Ramanujam, S., Lindner, D. J., Kudaravalli, R. D., Freund, R., and Kalvakolanu, D. V. (1998). The polyoma virus T antigen interferes with interferon-inducible gene expression. *Proc. Natl. Acad. Sci. USA* **95,** 1085–1090.

Wu, C. (1995). Heat shock transcription factors: Structure and regulation. *Annu. Rev. Cell. Dev. Biol.* **11,** 441–469.

Yokosawa, N., Kubota, T., and Fujii, N. (1998). Poor induction of interferon-induced 2′,5′-oligoadenylate synthetase (2-5 AS) in cells persistently infected with mumps virus is caused by decrease of STAT-1α. *Arch. Virol.* **143,** 1985–1992.

Yokota, S., Yanagi, H., Yura, T., and Kubota, H. (1999). Cytosolic chaperonin is up-regulated during cell growth. Preferential expression and binding to tubulin at G_1/S transition through early S phase. *J. Biol. Chem.* **274,** 37070–37078.